UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/4
6 November 1973

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM[1/]

No. 4

DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM[2/]

by: A. Nordal,

Department of Pharmacognosy,
Institute of Pharmacy,
University of Oslo,
Oslo-3, Norway.

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

GE. 73-12095

DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM

At the first Working Group in December 1972, I reported on the determination of the thebaine content of Papaver bracteatum. The analyses were carried out using the Sakurai method[3] as modified by B. Nielsen and J. Stene.[4]

Since that time, we have determined the thebaine content of the United Nations reference sample of P. bracteatum (UNB 6) and also of plant material raised in Norway from the seeds of P. bracteatum (UNB 2). Comparative analyses were carried out using the method of Fairbairn and Hakim and the method of Sakurai, as modified by Nielsen and Stene, and the results are given in Table 1.

The method of Fairbairn and Hakim was used in its original form as described by the authors in J. Pharm. Pharmacol., (1973), 25, 353-358. The Nielsen and Stene modification of Sakurai's method is given below.

Procedure

Mix 1 g of finely powdered sample with about 25 g of aluminium oxide (standardized activity II-III, Merck) and 2 ml of 15% sodium hydroxide solution. Transfer the uniform mixture quantitatively to a 18 x 220 mm chromatographic column. Elute the thebaine base with 200 ml of chloroform, at a rate not exceeding 2 ml per minute. Collect the eluate in a 500 ml round bottom flask and evaporate in vacuum to about 10 ml. Add 60 ml of 3.5% hydrochloric acid and heat and swirl until the chloroform is completely evaporated. 5 ml of the sample solution is placed in a 25 ml measuring flask and warmed in a boiling water bath for 25 minutes. When cooled, 4 ml of 0.5% 4-aminophenazone solution and 1.5 ml of 1% potassium ferricyanide solution are added and the mixture is shaken. After exactly one minute, 2 ml of 10% ammonia water is added, shaken again and warmed in a boiling water bath for 5 minutes.

The mixture is cooled with tap water to room temperature, diluted with 2.5 ml of ethanol and water to 25 ml. The absorbance of the solution is measured - after filtering - at 435 mμ.

3/ Sakurai, H., J. Pharm. Soc. Japan, (1960), 80, 909

4/ Of Weiders Pharmaceutical Company, Hausmannsgt.6, Oslo-1

Standard solution: Prepare a solution of pure thebaine base in 3.5% hydrochloric acid to give a concentration of 0.20 - 0.25 mg per ml. 5 ml of this solution is warmed for 25 minutes and the reagents added, as described above.

The blank test is carried out with the same reagents and in the same manner, using 5 ml of dilute hydrochloric acid instead of the test solution.

Table 1

Samples	Thebaine content in percentage, calculated on a dry weight basis according to:	
	Sakurai method as modified by Nielsen and Stene	Fairbairn - Hakim method
UN reference sample (powdered capsules) UNB 6	1.26	1.39
P. bracteatum cultivated in Norway from UNB 2 seeds		
Root	0.76 (0.39) x/	0.79
Leaves	0.80 (0,18) x/	0.81
Stem	0.58	0.77
Capsules	1.27	1.39

x/ The figures in parenthesis show the percentage of thebaine found in the young plants in the fall 1972.

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/5
8 November 1973

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM[1/]

No. 5

QUANTITATIVE ANALYSIS OF PAPAVER BRACTEATUM[2/]

by: Ping Cheng and Norman J. Doorenbos,

Department of Pharmacognosy,
Research Institute of Pharmaceutical Sciences,
School of Pharmacy,
University of Mississippi,
University, Miss. 38677,
United States of America.

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

GE. 73-12097

QUANTITATIVE ANALYSIS OF PAPAVER BRACTEATUM

The procedures which we are now following for the quantitative analysis of P. bracteatum for thebaine and isothebaine are as follows:

Plant material is dried to constant weight at 105° in a drying oven. Larger roots are cut into pieces 1 cm long prior to drying. Plant material is then ground by mortar and pestle to pass through a 0.5 mm sieve. The ground plant material is mixed thoroughly, with the aid of a spatula, before a sample is selected for analysis.

A 100 mg sample is weighed and the sample is extracted at room temperature twice with 30 ml portions of 3% acetic acid. The mixture is stirred 20 minutes with a magnetic stirrer during each extraction. Extracts are separated by filtration, the marc washed with an additional 10 ml of 3% acetic acid after the second extraction, and the extracts and washing are combined.

This solution is extracted three times with 20 ml portions of hexane. The pH of the aqueous solution is raised to 9 by the addition of sodium carbonate. The resulting mixture is extracted three times with 40 ml portions of ethyl ether. The combined ether extracts are washed once with 30 ml of water. The ether is evaporated, in vacuo, until the volume is about 10 ml. The ether solution is transferred to a 25 ml round bottom flask for final drying. The weight of the residue is then determined. This residue may be used for GLC or TLC studies.

GLC studies are being conducted with a Varian model 1200 gas chromatograph equipped with a hydrogen flame detector. A seven foot 1/8 inch stainless steel column packed with 3% OV-17 on 100/200 mesh Gas Chrom Q is being utilized. The carrier gas is nitrogen at a flow rate of 40 or 50 ml per min. Programmed and isothermal procedures have been utilized. The attenuation is set at 64×10^{-11} allowing the detection of .01 µg. of alkaloid. Sensitivity can be increased further, if needed. Routine analyses are being run isothermally at a column temperature of 260°. The inlet and detector are kept at 290°. A one or two µl. sample was normally injected. Peak area measurements were made using the method of peak height times width at half-height. The peak area of each alkaloid was compared with the peak area of the internal standard. Using the appropriate correction factors, the alkaloid concentration was then determined.

The new internal standard adopted is cholesterol acetate (1.0). Relative retention times of interest are thebaine (0.60), isothebaine (0.84) and cholesterol acetate (1.0). The internal standard is dissolved in n-butanol and kept in a tightly sealed container at room temperature. n-Butanol was selected as a solvent because its high boiling point would minimize evaporation and changes in concentration. Fresh solutions were made every two months, although no changes in concentration were detected. Calibration curves between the internal standard, cholesterol acetate, and the alkaloids, thebaine and isothebaine were made and checked every week. A standard sample of thebaine or P. bracteatum extract was run every day that analyses were made.

A series of drying experiments were conducted. Cut root pieces dry to constant weight in 12 hours at 105° and in 24 hours at 60°. Leaves dry even more rapidly. All dried plant parts pick up moisture if exposed to the air. Thus, samples should be freshly dried or stored in a dry, air-tight container, prior to analysis.

No difference in analysis could be detected between drying samples at 60° and 105°, if dried to constant weight. Thus, we now dry the samples overnight at 105°.

ST

UNITED NATIONS

SECRETARIAT

Distr.
GENERAL

ST/SOA/SER.J/6
6 December 1973

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM [1]

No. 6

ASSAY OF THEBAINE IN PAPAVER BRACTEATUM [2]

by: K.C. Reid, [3]
Technical Development Department,
Macfarlan Smith Ltd.,
Edinburgh, United Kingdom

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

3/ This paper was submitted by Dr. E.L. Grew, Research and Development, Macfarlan Smith Ltd.

GE.73-12378

ASSAY OF THEBAINE IN P. BRACTEATUM

A simple method for the assay of thebaine in Papaver bracteatum is required in order to cope with the large number of samples arising from a programme of breeding and selection work connected with the attempt to develop an improved variety suitable for the production of thebaine on a commercial basis. For this purpose most of the assays described in the literature are too tedious and time-consuming, requiring in many cases prior separation of thebaine from the other alkaloids of the poppy. Gas-liquid chromatography has been shown to give good results but is also time-consuming. A colorimetric method similar to the Guarino assay for morphine, which can be adapted for use in an AutoAnalyzer, is considered most suitable. Colorimetric assays for thebaine described in the literature depend on conversion of thebaine to the phenolic thebenine which is then determined by a variety of colour reactions based on its phenolic nature. These methods are thus susceptible to the presence of other phenolic materials and do not claim specificity for thebaine. Even when phenols are removed by an ion exchange procedure prior to conversion of thebaine to thebenine, many poppy samples were shown to contain other readily-oxidisable substances which gave colour reactions with phenol reagents.

A differential procedure has been devised whereby the poppy sample is extracted with cold, very dilute acid, two aliquots of the clarified extract taken, the first treated with strong hydrochloric acid and heated for a period sufficient to convert the thebaine to thebenine, the second similarly treated with acid but not heated. Both are then subjected to a colorimetric assay of phenolic material such as that described by Sakurai, J. Pharm. Soc. Japan, 1960, 80, 909, the difference representing the thebaine content of the sample. In this way the effect of initial colour of the sample and also of other substances which give colour reactions with the phenol reagent are eliminated, the only interference being from substances which may be converted to colour-producing substances by the period of heating alone. There is no evidence that such substances are present in the poppy samples tested to an extent sufficient to affect the usefulness of this method as a screening procedure.

Procedure for manual operation

The dry poppy sample is milled through a 20 mesh screen and 0.25 gramme weighed out into a 20 ml vial with a polythene closure. 10 ml of distilled water is added together with 1 ml of normal hydrochloric acid. The vial is then shaken vigorously on a mechanical shaker for 30 minutes and the slurry filtered through Whatman 42 papers without washes. 1 ml of the clear filtrate is pipetted into each of two 20 ml vials and 1 ml of 2N hydrochloric acid added to each. One only of these vials (loosely capped to avoid loss by evaporation) is placed in a hot water bath at 95 to 100°C for 25 minutes, then cooled. To each vial is then added 1.5 ml of 0.5% 4-aminoantipyrene aqueous solution and 0.6 ml of 1% potassium ferricyanide aqueous solution. After shaking for exactly 1 minute, 2 ml of 10% ammonia aqueous solution is added to each and the vials shaken and placed in the hot water for 5 minutes. After cooling, 1 ml of ethanol is added to each vial and the total volume made up to 10 ml with water. The solutions should be clear at this stage but if the concentration of thebaine in the original acid extract is more than 200 mg/L (about 1% in the sample tested) a brown flocculent precipitate may gradually appear, rendering the solution turbid and the result invalid. In such cases, the assay must be repeated after diluting the acid extract by a factor of 2 (for samples containing up to 2% thebaine) or proportionately more for samples of higher thebaine content. If clear, the absorbance of each solution is read at 435 mμ.

The nett absorbance (of the heated sample less that of the unheated sample) is compared with a calibration chart made by carrying out the above procedure using a series of standard solutions of thebaine hydrochloride ranging in thebaine content from 50 to 200 mg per litre in place of the filtered poppy extract. The graph approximates to a straight line through the origin having a slope of about 60 mg/L per 0.1 absorbance. This gives the thebaine content of the poppy extract in mg/L and this figure multiplied by 0.0044 gives the thebaine content of the poppy sample as a percentage. If any absorbance reading exceeds 0.6 or if the nett absorbance exceeds 0.4, the assay should be repeated after diluting the poppy extract by an appropriate factor to reduce the absorbance below this figure, the result being correspondingly corrected. Many samples are found

to give very high 'blank' readings, particularly samples of leaves etc containing chlorophyll and the accuracy of the assay is therefore poorer for those than for samples (of root or capsule for example) giving low 'blanks'. Similarly, low results are more subject to inaccuracies than high ones and figures indicating less than 0.1% thebaine in the poppy should not be taken as significant.

Adaptation for AutoAnalyzer use

For use with an AutoAnalyzer, the method can be considerably streamlined. The first heating stage may be modified from 25 minutes at 100°C to 25 minutes at 80°. The second period of heating may also be reduced to 80° or even omitted altogether. Final filtrations are not usually necessary except with samples of very high absorbance, which should in any case be repeated at a lower concentration. The use of ethanol may be omitted and the concentration of the reagents may be varied to give the correct final volume without final adjustment with water. Results produced by the AutoAnalyzer adaptation of the assay are more reproducible than those obtained by hand, as delays and agitation stages are standardised and, except in the circumstances mentioned above, the absorption readings completed before any turbidity in the final solution has time to develop.

This modified version of the Sakurai assay has been found to give results which are in reasonably good agreement with those obtained by GLC, as shown in the table below:

		% thebaine on dry weight	
Sample	Ref. No.	Modified Sakurai	G.L.C.
Root	1972/5A	0.65	0.66
Root	1972/5B	0.72	0.61
Root	1972/5C	0.55	0.61
Root	1972/6A	0.51	0.59
Root	1972/6B	0.60	0.59
Root	1972/6C	0.56	0.69
Root	1972/7	0.07	0.01
capsule, stem, root	P31973/30 (1A)	0.30	0.30
do	P31973/31 (1A)	0.24	0.28
do	P31973/32 (2B)	0.20	0.18
do	P31973/33 (2B)	0.14	0.14
capsule	P31973/30A	1.6	1.9

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/7
7 December 1973

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM 1/

No. 7

DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM 2/

by: I. Lalezari,

Department of Organic Chemistry,
Faculty of Pharmacy,
University of Teheran,
Teheran, Iran

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated, to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

GE.73-12380

DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM

Plant material

The yellow-green capsules collected just prior to maturation were found to contain the maximum of thebaine. Capsules were dried in the open air in the shade. Seeds were separated and the residue was dried at 60° to constant weight and finely ground.

U.V. Spectrophotometric determination of thebaine

A mixture of 1.0 g of powdered capsules and 20 ml of benzene containing 2 ml of concentrated ammonia was stirred for one hour at room temperature. The mixture was filtered with suction and the residue was stirred with 20 ml of benzene for one hour and filtered. The residue was washed with 10 ml of benzene. The combined extracts and benzene washings were evaporated under reduced pressure. The residue was dissolved in 0.1 N HCl and diluted with the same solvent to 500 ml (a small amount of charcoal may be used in the case of coloured solutions). The extinction at 285 mμ (max.) was measured using the E 1%, 1 cm value of 250.

Gravimetric method

The residue from the evaporation of the benzene extracts of 10.0 g of powdered capsules (using a total of 100 ml of benzene and 5 ml of concentrated ammonia) was dissolved in 50 ml of 0.1 N HCl and extracted with three 15 ml portions of ether to remove the colouring matter. To the aqueous layer, concentrated ammonia was added until the reaction was alkaline and three 10 ml portions of chloroform were used for the extraction. After evaporation of the solvent, the crystalline residue was recrystallized from 96% alcohol. Using either chloroform or methanol instead of benzene gave identical results. Also, a 50% aqueous solution of sodium carbonate could be used instead of ammonia.

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/8
27 March 1974

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM [1]

No. 8

ANALYSIS OF PAPAVER ALKALOIDS AND STABILITY OF THEBAINE [2]

by: F.J.E.M. Küppers, R.J.J.Ch. Lousberg,
C.A.L. Bercht and C.A. Salemink,

Organic Chemistry Laboratory,
State University,
Utrecht, The Netherlands.

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories. It was also recommended that a study be made of the stability of thebaine.

GE.74-4226

Within the scope of our work on opium alkaloids, we became particularly interested in the botanical and chemical aspects of Papaver bracteatum. Detailed motives for this special interest are described in the first two reports in the United Nations series on scientific research on Papaver bracteatum[1a,b] and in the interesting article by Fairbairn and Hakim[2] on P. bracteatum Lindl., as a new source of opiates.

From the above reports, it was concluded t at immediate attention should be given inter alia to chemical aspects such as the extraction and analysis of P. bracteatum and the stability of thebaine, one of the major constituents of several races of P. bracteatum. The present study concerns these last aspects.

Chromatographic analyses of opiates

Gas chromatography. Several methods have been investigated for the qualitative and quantitative analysis of the alkaloids, such as those described by Brochmann-Hanssen[3], Nieminen[4], Cheng[5] and Jones[6]. It was considered important for good quantitative analysis that the morphine and thebaine should be well separated. The following compounds were used as internal standards: histapyrrodine hydrochloride, estradiol valerate and cholesterol acetate. The solid support was either 60-80 mesh or 100-120 mesh.

The best results with regard to reproducibility, separation and tailing were obtained with 2 per cent OV-17 on Chromosorb-G AW-DMCS 100-120 mesh at temperatures between 260-300°. It was found that the solid support of 60-80 mesh caused considerable tailing. The instrumental conditions were:

gas chromatograph	: Becker 409
detection	: F.I. and digital integrator
column	: 6 ft. glass, inner diameter 3 mm, 2 per cent OV-17 chromosorb-G AW-DMCS, 100-120
injector temperature	: 290°
detector temperature	: 310°
oven temperature	: 25 min. isotherm at 270°, next 5°/min. until 290° and finally isotherm at 290°
carrier gas	: nitrogen, 2.5 atm. inlet pressure, flow rate approx. 45 ml/min.

A sample of P. bracteatum (UNB 6) was extracted by

(a) the method of Nieminen[4] (treatment with water at pH 10.0 and extraction with chloroform. The combined chloroform layers were dried over $MgSO_4$, evaporated to dryness and re-dissolved in methanol)
(b) the method of Fairbairn[2] (extraction with ammoniacal methanol, evaporation to dryness and re-dissolving in methanol).

Under the above gas chromatographic conditions, the sample analysed as indicated in Table I.

Table I

Sample UNB 6	Retention time in min. (RT)	Relative retention time (RRT)	Relative integration value from integrator counts
M^+ m/e 313;298	8.4	0.45	0.02
M^+ m/e 254;239	12.6	0.67	0.04
Thebaine	18.0	1.00	1.00
M^+ m/e 297	22.0	1.20	0.04

Thebaine was found to be present in a concentration of 0.4 per cent. It seems that the method of extraction is of great importance. The investigations are continuing and further details will be presented in a forthcoming report.

Thin layer chromatography. Different tlc systems were used e.g. the methods of Fairbairn[2] (SiO_2 and benzene-methanol 4:1), the standard system "S 1" of Pfeiffer[7] (SiO_2 and benzene-acetone-methanol 7:2:1) and the method described by Stahl[8] (SiO_2 and toluene-acetone-ethanol-ammonia 25 per cent 40:40:6:2). Detection of the alkaloids was carried out with the iodoplatinate spray reagent[9]. Fertigplatten Kieselgel Merck were used.

Comparison of the above systems clearly showed the first two to be less favourable, because of the unavoidable tailing of the different spots. The system of Stahl gave excellent results and was further used during the following experiments. After one run, the Rf-values for morphine, codeine, thebaine and papaverine were 0.12, 0.20, 0.35 and 0.60, respectively, with the following colour reactions immediately after spraying: gray-violet, violet, blue-violet and red-violet and, after several hours, brown-violet, violet, brown-violet and yellow, respectively.

Analysis of thebaine samples and choice of material. Thebaine*/ and its hydrochloric acid salt**/ were first analysed using the above chromatographic systems. Both products were in white crystalline form.

	GLC RRT.	%	TLC Rf.
Thebaine	0.96	0.2	-
	1.00	99.8	0.35
Thebaine HCl	0.96	0.1	-
	1.00	99.9	0.35

It has been mentioned by Brochmann-Hanssen[10] that both thebaine and its HCl-salt can be used in gas chromatography. Decomposition of the salt in the injector gives thebaine as indicated by the above data. However, we found that the quantitative analysis of the salt is far less reproducible ($\pm$ 30 per cent) than in the case of the free base ($\pm$ 5 per cent). This observation can certainly be ascribed to variations in the degree of decomposition of the salt. For quantitative thebaine analysis, it is advisable to adjust the aqueous thebaine-salt solutions to pH 9 with ammonia and to extract at least twice with chloroform.

The optimal response in the gas chromatographic analysis of thebaine was obtained under the following conditions:

electrometer : range 1, attenuation 16
flame : H_2 pressure 0.75 kg/cm^2
air pressure 2.00 kg/cm^2
carrier gas : N_2 flow rate 45 ml/min.
column temperature : 270°

The absolute response towards 1 µg thebaine was 4.8 cm^2 peak area.

---

*/ Obtained from the Nederlandse Fabriek voor Farmaceutische Producten, The Netherlands.

**/ Obtained from the Onderlinge Farmaceutische Groothandel, The Netherlands.

Table II

	Retention time in min. (RT)	Relative retention time (RRT)	Relative integration value from integrator counts
Codeine HCL	11.0	0.61	0.8
Morphine	13.3	0.74	1.0
Thebaine	18.0	1.00	1.3
Papaverine	32.0	1.78	0.9

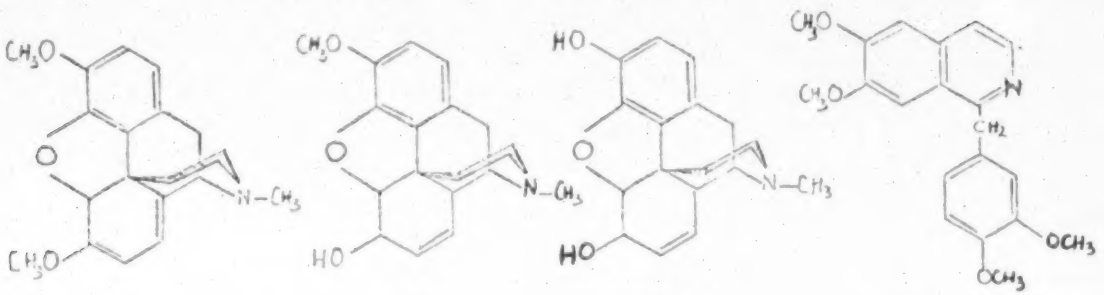
Combined gas chromatography-mass spectrometry of opiates. Analysis of unknown opiates or degradation products in stability experiments can only effectively be carried out by methods which provide both quantitative and qualitative information. Therefore, in addition to the above chromatographic methods, we studied the application of combined gas chromatography-mass spectrometry (GCMS). The experiments were carried out with a JEOL-1100 gas chromatograph equipped with a modified JEOL JMS-07 mass spectrometer (with a double stage jet separator) for the detection.

For orientation, the following substances were first measured: morphine, codeine, heroin, thebaine, narcotine, narceine, papaverine and protopine.

The conditions were as follows:

column : 3 per cent OV-17, Chromosorb-G AW-DMCS
column temperature : 225°
pre-pressure : He, 1.05 atm.
source temperature : 230°
accelerating voltage : 3.0 kV
trap current : 300 µA
electron energy : 70 eV

Characteristic fragment ions and their relative intensities of the four major opiates thebaine (I), codeine (II), morphine (III) and papaverine (IV) are:



I II III IV

I II III IV

I. M/e 311 (100%), 296 (58%), 282 (5%), 280 (4%), 268 (7%), 267 (8%), 265 (4%), 254 (8%), 253 (6%), 250 (3%), 242 (12%), 239 (10%), 211 (9%), 182 (6%), 165 (7%), 58 (14%), 44 (10%), 42 (44%).

II. m/e 299 (65%), 298 (6%), 284 (4%), 282 (6%), 242 (5%), 229 (25%), 214 (15%), 188 (18%), 162 (45%), 124 (32%), 115 (24%), 96 (7%), 94 (12%), 91 (10%), 81 (26%), 59 (38%), 44 (48%), 42 (100%).

III. m/e 285 (100%), 270 (4%), 268 (14%), 256 (5%), 242 (4%), 228 (7%), 215 (34%), 174 (17%), 162 (42%), 124 (30%), 115 (18%), 70 (26%), 44 (48%), 42 (76%).

IV. m/e 339 (32%), 338 (35%), 324 (39%), 315 (6%), 308 (10%), 220 (100%), 205 (16%), 154 (8%), 147 (7%), 91 (8%), 77 (10%).

These products can be well recognized by their molecular weight and they possess molecular ion peaks of moderate to strong intensity.

Stability experiments

Preliminary stability experiments. At first, relatively vigorous conditions were applied. Thebaine-HCl (10 mg) dissolved in 0.5 ml of:

(a) 0.5 N HCl

(b) 1 M tartaric acid

(c) 2% H_2SO_4

was kept for 24 hr at 95-100° in sealed tubes.

Free thebaine (10 mg) suspended in 0.5 ml of:

(d) 0.5 N NaOH

(e) saturated Na_2CO_3 solution

(f) 10% NH_3/H_2O

was treated under similar conditions.

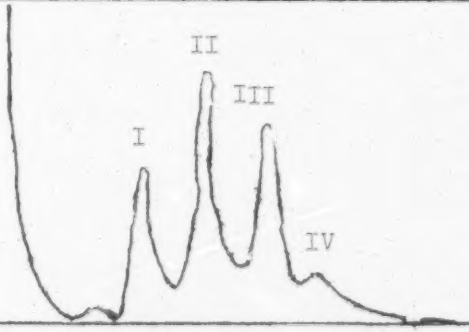
After 24 hours, the acidic reaction mixtures were adjusted to pH 9-10 with ammonia and all reaction mixtures were extracted with chloroform. The chloroform was evaporated and the residue dissolved in methanol. GLC and TLC analyses of the methanolic solutions revealed that thebaine was resistant towards treatments (d), (e) and (f). Treatments (a), (b) and (c) converted thebaine quantitatively into at least four different products.

Stability of thebaine towards 0.01 N HCl. Thebaine-HCl was dissolved in 10 mg amounts in 0.2 ml 0.01 N HCl in sealed tubes (0.5 x 10 cm). Two series of experiments were made:

A. at 90° for (1) 1 hr; (2) 2.5 hr; (3) 5 hr; (4) 8 hr; (5) 12 hr

B. for 12 hr at (1) 30°; (2) 40°; (3) 50°; (4) 60°; (5) 70°; (6) 80°.

Sample A5 was analysed by GCMS and the results are given in the following table.

Total ion current chromatogram	Characteristic mass fragments and their relative intensities
	I. 313 (100%); 298 (15%); 296 (20%); 256 (70%); 255 (65%); 223 (45%) II. 311 (12%); 296 (8%); 280 (45%); 265 (100%); 250 (20%); 235 (30%) III. 293 (100%); 278 (65%); 265 (20%); 250 (40%); 235 (50%) IV. 291 (15%); 276 (10%)*/
RRT 0.76/1.00/1.30/1.61	

*/ These data are difficult to interpret due to the tailing of product III.

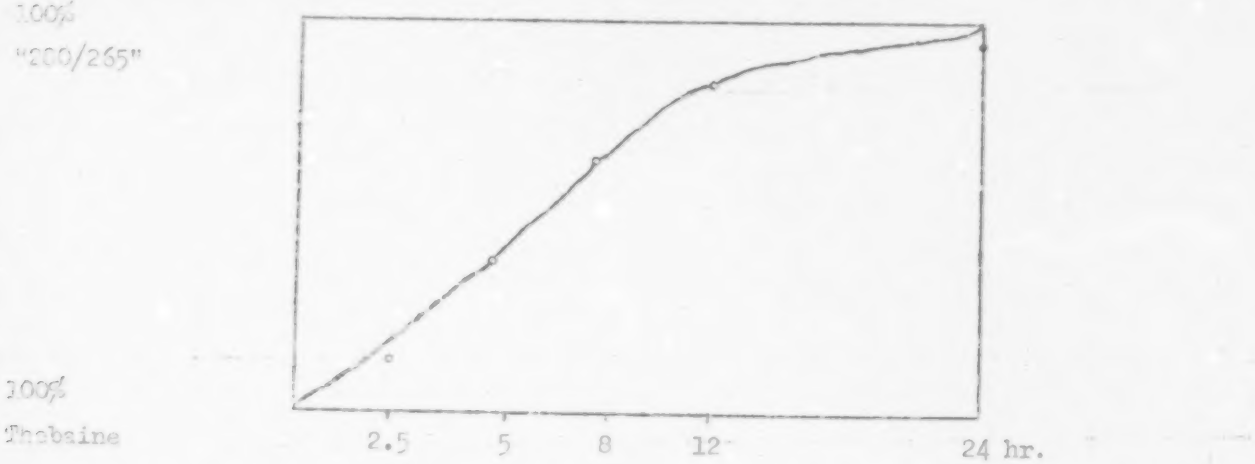
TLC analysis of sample A5 indicated the presence of a weak spot with the Rf-value corresponding to thebaine (0.35) and three further main products with Rf 0.03 (violet with iodoplatinate), 0.72 (light-yellow) and 0.80 (light-yellow). The peak in the ion current chromatogram with RRT 1.00 could, therefore, not correspond to thebaine only.

The same conclusion is provided by comparison of the mass spectrum of peak II in A5 with that of thebaine:

Thebaine	311 (100%); 296 (60%); 280 (4%); 265 (3%); 250 (3%)
Peak 2 in A5	311 (12%); 296 (8%); 280 (45%); 265 (100%); 250 (20%)

After a longer reaction time (24 hr) and at a higher reaction temperature (95°) it was found that the peak with RRT 1.00 consisted only of a product with M^+ m/e 280 and with the base peak at m/e 265. Thus, the product seems to lack the nitrogen atom. Further study is in progress.

The relation between reaction time and the ratio thebaine: compound "280" in peak II at a temperature of 90° in a 0.01 N HCl solution is given in the following figure. The ratio was determined by comparison of the intensities of the base peak of thebaine at m/e 311 with that of the "280" compound at m/e 265.

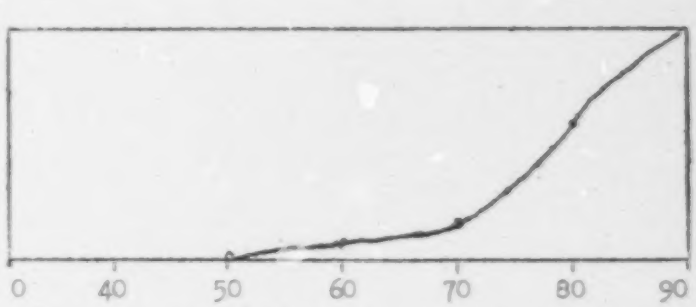


Next to the formation of the "280/265" product, three more substances could be analysed: "313/298", "293/278" and "291/276". The relative formation of these products after different reaction times is given in the next table.

Temperature 90°	1 hr	2.5 hr	5 hr	8 hr	12 hr
"313/298"	-	2%	2.5	3	4
"293/278"	-	4	13	25	40
"291/276"	-	1	1.5	2.5	3
"280/265"	5	12	21	34	44
Thebaine	95	81	62	33.5	9

Stability of thebaine at lower temperatures. The experimental series B with regard to the stability of thebaine towards 0.01 N HCl gave results which can be summarized in the following graph:

% of decomposition products relative to starting material after 12 hr reaction time



The above graph clearly illustrates that, during at least 12 hr, thebaine is stable in 0.01 N HCl at temperatures below 50°.

Stability of thebaine towards several acids used in opiate-processing. The stability of thebaine was investigated towards 0.01 N HCl, 0.1 M tartaric acid, 0.1 M citric acid and 0.5 M acetic acid at 80 and 90° after 12 hr reaction time and addition of a solution of 0.1 M $NaHSO_3$.

Reaction time 12 hr						+ 0.1 M $NaHSO_3$				
	Relative conc. of products					Relative conc. of products				
	I	280	thebaine	III	IV	I	280	thebaine	III	IV
0.01 N HCl 80°	3	36	37	22	2					
0.01 N HCl 90°	4	44	9	40	3	7	28	59	4	2
0.1 M tartaric acid 80°	2	41	48	8	1					
0.1 M tartaric acid 90°	6	49	10	33	2	6	-	94	-	-
0.1 M citric acid 80°	4	1	95	-	-					
0.1 M citric acid 90°	10	42	28	16	4	4	-	96	-	-
0.5 M acetic acid 80°	2	-	98	-	-					
0.5 M acetic acid 90°	4	2	94	-	-	2	-	98	-	-

The results given in the above table clearly indicate the stabilizing effect of the addition of $NaHSO_3$ to the reaction mixture. This is particularly so in the case of the treatment of thebaine with tartaric acid and with citric acid.

Stability of thebaine and its HCl-salt towards oxygen and light. The possible influence of oxygen and light on methanolic solutions of thebaine and its HCl-salt was studied.

During 12 hr, oxygen was bubbled through a solution of thebaine (15 mg) in methanol at a flow rate of 10 ml/min. One solution was kept at room temperature in the dark, a second solution was exposed to light of two TL-tubes at a distance of 40 cm. Neither of the solutions showed measurable decomposition of the thebaine.

The same experiments were carried out with an aqueous solution of thebaine HCl-salt. In the dark, no decomposition was observed. However, under the influence of light, at least 10 per cent of the thebaine salt was converted into the product with typical mass fragments "313/298".

Further experiments:

1. Oxygen (10 ml/min) was bubbled through a solution of 15 mg thebaine HCl in 0.5 ml undistilled water (pH 6) at 80° for 12 hr. Light was provided by two 65 W TL-tubes at a distance of 40 cm.
2. The same experiment was carried out at 25°, using a 0.001 N HCl solution.
3. Similar as for 2, using a 0.01 N NaOH solution.

Results:

Exp.	RRT	%	MS data
1.	0.52	2	-
	0.76	14	"313/298"
	1.00	82	thebaine
	1.30	2	"293/278"
2.	0.76	3	"313/298"
	1.00	97	thebaine
3.	1.00	99	thebaine

Conclusions

GCMS provides valuable information in the analysis of opiates. Thus it was found that thebaine-HCl could be converted at 90° into a major product possessing identical retention time as thebaine itself. However, from its mass spectrum, it was clearly demonstrated that this product was different from thebaine.

Experiments with regard to the stability of thebaine indicated that:

(a) the temperature to which thebaine is exposed is of great importance;

(b) the pH of the preparation influences the degree of decomposition;

(c) working temperatures below 50° cause only slight decomposition;

(d) addition of $NaHSO_3$ during working processes with thebaine provides considerable stabilization;

(e) oxygen and light affect solutions of thebaine hydrochloride.

References

(1) (a) United Nations document ST/SOA/SER.J/1 (1973)
(b) United Nations document ST/SOA/SER.J/2 (1973)

(2) J.W. Fairbairn and F. Hakim, J. Pharm. Pharmac., 25, 353 (1973)

(3) E. Brochmann-Hanssen and T. Furya, J. pharm. Sci., 53, 1549 (1964)

(4) E. Nieminen, Farm. Aikak., 80, 342 (1971)

(5) P.C. Cheng, Thesis, University of Mississippi (1972)

(6) Q. Jones and W.A. Gentner, Communication to the United Nations Narcotics Laboratory (1973)

(7) S. Pfeiffer, J. Chromatogr., 41, 127 (1969)

(8) E. Stahl, H. Jork, E. Dumont, H. Bohrmann and H. Volmann, Arzneimittel-Forsch., 19, 194 (1969)

(9) R. Munier, Bull. Soc. chim, Fr., 19, 852 (1952)

(10) E. Brochmann-Hanssen and A. Baerheim Svendsen, J. pharm. Sci., 51, 1095 (1962)